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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07K 5/02, 5/06, 5/08 C07K 5/10, 7/02, 7/06 C07K 7/08	A1	(11) International Publication Number: WO 91/02745
		(43) International Publication Date: 7 March 1991 (07.03.91)

(21) International Application Number: PCT/US90/04633

(22) International Filing Date: 16 August 1990 (16.08.90)

(30) Priority data:
394,727 16 August 1989 (16.08.89) US

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(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), HU, IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: THERAPEUTIC PEPTIDES

(57) Abstract

A linear peptide which is an analog of naturally occurring, biologically active substance P having an active site and a binding site responsible for the binding of said peptide to a receptor on a target cell. The analog has a non-peptide bond instead of a peptide bond between an amino acid residue of said active site and an adjacent amino acid residue, or a synthetic, a β-amino acid, or a γ-amino acid residue replacing two amino acid residues of the active site.

* See back of page

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THERAPEUTIC PEPTIDES

Background of the Invention

This invention relates to therapeutic peptides, in particular analogs of the naturally occurring peptide substance P.

Substance P (SP) has numerous pharmacologic effects including vasodilation and hypotension, contraction of non-vascular smooth muscle, stimulation of salivary and pancreatic secretion, depolarization of various neurons and histamine release from mast cells. SP is thought to play a variety of physiological roles (many of which are associated with the induction of pain). These include regulation of peristalsis and smooth muscle activity in the gastrointestinal tract, regulation of salivary and pancreatic secretion, regulation of the inflammatory response to peripheral tissue injury, neurotransmission, and regulation of neuro-immunomodulation. Mantyh et al. (1989) Proc. Natl. Acad. Sci. USA 86:5193 reports the presence of substance P receptors at wound sites in the central nervous system and suggests that SP may be involved in regulating the response to injury in the central nervous system as well as in peripheral tissues. Substance P is also a proliferative agent, stimulat the proliferation of fibroblasts, T-lymphocytes, e. :helial cells, smooth muscle cells and astrocytes.

SP belongs to a family of bioactive peptides known as the tachykinins. The structure, activity, and function of SP and other tachykinins are discussed in Payan (1989) Ann. Rev. Med. 40:341. As discussed in Payan, SP shares common pharmacological properties and a conserved carboxyl terminal sequence (Phe-X-Gly-Leu-Met-NH_2, where X is a branched aliphatic or aromatic amino acid residue) with the

other tachykinins. The principle biological activities, and the ability to bind to a receptor, reside in the carboxyl terminal sequence of these peptides. Selectivity toward a specific tachykinin receptor is determined by the amino-terminal sequence of the peptides Iverson et al., (1989) The Tachykinin System, Abstract presented at the 11th American Peptide Symposium. The conservation of carboxyl terminal sequence extends beyond SP and other mammalian tachykinins to other bioactive peptides, as shown in Table 1.

Numerous derivatives of SP, made by side chain modification and/or D-amino acid substitution, have been shown to act as SP receptor antagonists. Folkers, U.S. Patent No. 4,481,139, describes substance P antagonists made by D or L amino acid substitution. These antagonists include the undecapeptide analog spantide (D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp- Phe-D-Trp-Leu-Leu-NH_2) as well as truncated analogs of substance P. Jensen et al. (1988) Am. J. of Physiol. 254: G883 characterized the ability of various SP antagonists to inhibit the action of bombesin. Jensen et al. studied four SP analogues: Arg-D-Pro-Lys-Pro-Gln-Gln-D-Phe-Phe-D-Trp-Leu-Met-NH_2; Arg-D-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Met-NH_2; D-Arg-D-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2; and D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2. Jensen et al. also studied two SP analogues with the first 3 amino acid residues deleted, D-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Met-NH_2 and D-Pro-Gln-Gln-D-Trp-Phe-D-Trp-D-Trp-Met-NH_2. None of these receptor binding peptides are, however, specific to the SP receptor. All were found to inhibit bombesin-stimulated amylase release. Jensen et al. concluded that "the ability to inhibit the action of bombesin is a general property of SP analogues that also function as SP receptor antagonists and that the SP receptor antagonists are each inhibiting the action of bombesin by functioning as bombesin receptor antagonists."

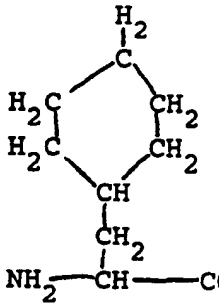
Woll et al. (1988) Proc. Nat. Acad. Sci. USA 85: 1857 found the substance P antagonist D-Arg-Pro-Lys-Pro-D-Phe-Gln- D-Trp-

Phe-D-Trp-Leu-Leu-NH_2 a potent bombesin antagonist in murine Swiss 3T3 cells.

Agonists and antagonists of a wide spectrum of biologically active peptide hormones, including substance P, have been synthesized by the introduction of modification of the peptide bonds of the peptide hormone, see Spatola (1983) in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins (B. Weinstein, ed.) M. Dekker, New York and Basel, pp. 267-357, for a recent review of the field.

Abbreviations (uncommon):

cyclohexyl-Ala = (cyclohexyl alanine)

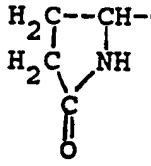


identifying group

Lys-ϵ-NHR = lysine wherein the ϵ-N atom carries an R group (where R is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE (where E is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or C_1-C_{12} acyl);

Nle = H_2N-CH-COOH (norleucine)
$(CH_2)_3$-CH_3 identifying group

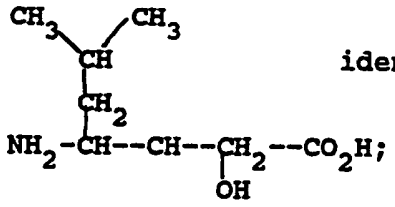
Nal = naphthylalanine

pGlu=  (pyroglutamic acid)

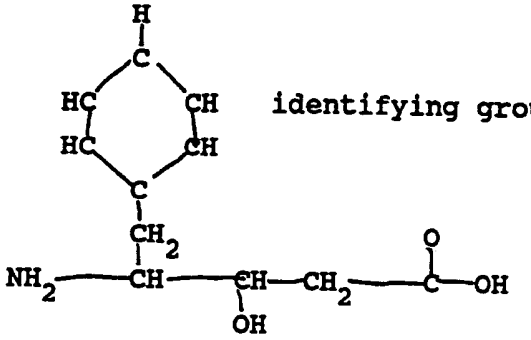
Sar = sarcosine

Sta (statine) =

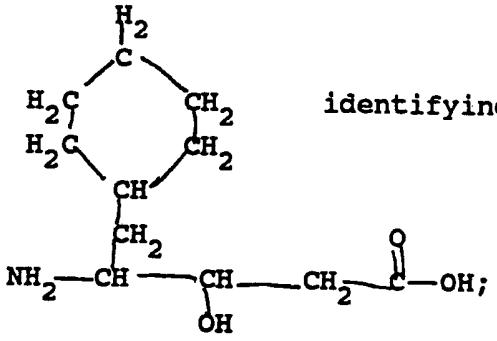
(3S, 4S)-4-amino-3-hydroxy-6-methylheptanoic acid, and has the chemical structure:



AHPPA =
(3S,4S)-4-amino-3-hydroxy-5-phenylpentanoic acid, and has the chemical structure:



ACHPA =
(3S, 4S)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid and has the chemical structure:



SP = Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (substance P).

Summary of the Invention

In general, the invention features a linear (i.e., non-cyclic) peptide which is an analog of naturally occurring, biologically active substance P having an active site and a binding site responsible for the binding of the peptide to a

receptor on a target cell. The analog has one of the following modifications: (a) a non-peptide bond instead of a peptide bond between an amino acid residue of the active site and an adjacent amino acid residue, (b) a replacement of two amino acid residues within the active site with a synthetic amino acid residue, e.g., statine, AHPPA, ACHPA, a ß-amino acid residue, or a γ-amino acid residue, (c) a deletion of an amino acid residue within the active site and a modification of an amino acid residue outside of the active site, or (d) the presence of an N-terminal amino acid residue that is not the naturally occurring amino acid residue of said naturally occurring, biologically active peptide (where ß- or γ- is not designated an amino acid is an α-amino acid).

In preferred embodiments the analog is capable of acting as a competitive inhibitor of naturally occurring substance P by binding to the receptor and, by virtue of one of the modifications, failing to exhibit the in vivo biological activity of the naturally occurring peptide.

In preferred embodiments the active site of the linear peptide is in the carboxyl terminal-half of the linear peptide. The linear peptide has one of the following modifications: (a) a non-peptide bond instead of a peptide bond between an amino acid residue of the active site and an adjacent amino acid residue, (b) a replacement of two amino acid residues within the active site with a synthetic amino acid residue, e.g., statine, AHPPA, ACHPA, a ß-amino acid residue, or a γ-amino acid residue, (c) a deletion of an amino acid residue within the active site and a modification of an amino acid residue outside of the active site, or (d) the presence of an N-terminal amino acid residue that is not the naturally occurring amino acid residue of said naturally occurring, biologically active peptide.

In preferred embodiments the active site of the linear peptide is in the carboxyl terminal-half of the linear peptide. The linear peptide has one of the following modifications, (a) a non-peptide bond instead of a peptide bond between the carboxyl

terminal amino acid residue and the adjacent amino acid residue, or (b) a statine or AHPPA or ACHPA, ß-amino acid, or γ-amino acid residue in place of the naturally occurring carboxyl terminal and adjacent amino acid residues.

In preferred embodiments the analog of substance P has one of the following modifications, (a) a non-peptide bond instead of a peptide bond between the carboxyl terminal amino acid residue and the adjacent amino acid residue, or (b) a statine or AHPPA or ACHPA, ß-amino acid, or γ-amino acid residue in place of the naturally occurring carboxyl terminal and adjacent amino acid residues.

In preferred embodiments the linear peptide is an analog of substance P with an active site in the carboxyl terminal-half of the linear peptide. The analog of substance P has one of the following modifications: (a) a non-peptide bond instead of a peptide bond between the carboxyl terminal amino acid residue and the adjacent amino acid residue, or (b) a statine or AHPPA or ACHPA or ß-amino acid or γ-amino acid residue in place of the naturally occurring carboxyl terminal and adjacent amino acid residues.

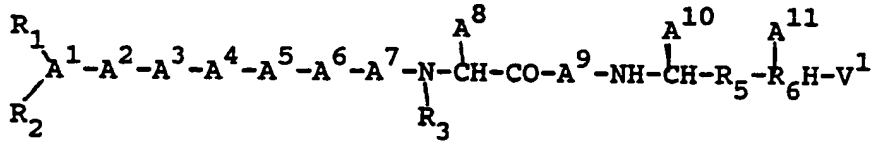
The linear peptides for which introduction of a non-peptide bond between two amino acid residues, or the replacement of two natural amino acid residues with a synthetic amino acid residue, a ß-amino acid residue, or a γ-amino acid residue, or the deletion ("des") of the C-terminal amino acid residue useful in creating or enhancing antagonist activity are those in which activity is associated with the two C-terminal amino acid residues of the amino acid chain. Therefore, the active site of the naturally occurring peptide of which the peptides of the invention are analogs preferably includes at least on amino acid residue in the carboxy terminal half of the peptide, and the linear peptide of the invention includes that amino acid residue in its carboxy terminal half. Modifications can be introduced in a region involved in receptor binding, or in a non-binding region.

By non-peptide bond is meant that the carbon atom participating in the bond between two residues is reduced from a carbonyl carbon to a methylene carbon, i.e., CH_2-NH; or, less preferably, CH_2-S, CH_2-CH_2, CH_2-CO, or CO-CH_2. (A detailed discussion of the chemistry of non-peptide bonds is given in Coy et al. (1988) Tetrahedron 44,3:835-841, hereby incorporated by reference, Tourwe (1985) Janssen Chim. Acta 3:3-15, 17-18, hereby incorporated by reference, and Spatola (1983) in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, (B. Weinstein, ed.) M. Dekker, New York and Basel, pp. 267-357, hereby incorporated by reference.)

Preferably, analogs of the invention are 25% homologous, most preferably, 50% homologous, with the naturally occurring peptides.

One modification of the naturally occurring peptide to create an antagonist is to employ, as the amino terminal residue, an aromatic D-isomer of an amino acid, or an alkylated amino acid. (Where "D" is not designated as the configuration of an amino acid, L is intended.)

One class of peptide of the invention includes the substance P analogs of the formula:



wherein

A^1 = the D or L isomer of any one of the amino acids Arg, Lys, or Lys-ε-NH-R_{20} (where R_{20} is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_{10} (where E_{10} is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or C_1-C_{12} acyl); or is deleted;

A^2 = the D or L isomer of the amino acid Pro; or is deleted;

A^3 = the D or L isomer of any one of the amino acids Lys, or Lys-ε-NH-R_{22} (where R_{22} is any of H, C_{1-12} alkyl,

C_{7-10} phenylalkyl, COE_{12}^{8} (where E_{12} is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or C_1-C_{12} acyl); or is deleted;

A^4 = the D or L isomer of the amino acid Pro; or is deleted;

A^5 = the D or L isomer of any one of the amino acids Asp, Gln, ß-Nal, Trp, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3); or is deleted;

A^6 = the D or L isomer of any one of the amino acids Ala, Arg, Ser, Pro, Gln, pGlu, Asn, ß-Nal, Trp, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3);

A^7 = the D or L isomer of any one of the amino acids Val, Thr, Phe, Trp, ß-Nal, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3);

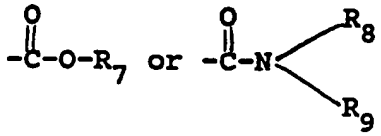
A^8 = the identifying group of the D or L isomer of any one of the amino acids Gly, Val, Trp, ß-Nal, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3);

A^9 = the D or L isomer of any one of the amino acids Sar, His, Gly, Trp, ß-Nal, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3);

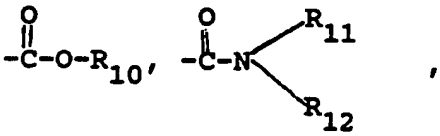
A^{10} = the identifying group of the D or L isomer of any one of the amino acids Trp, ß-Nal, Leu, Nle, Ala, cyclohexyl-Ala, Val, Ile, Met, Gly, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3);

A^{11} = the identifying group of the D or L isomer of any one of the amino acids Trp, ß-Nal, Leu, Nle, Ala, Val, Ile, Met, Gly, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3); or is deleted; each R_1 and R_2, independently,

is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_{14} (where E_{14} is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), C_1-C_{12} acyl, or is deleted, and R_1 and R_2 are bonded to the α-amino nitrogen atom of the N-terminal amino acid of said peptide, provided that, when one of R_1 or R_2 is COE_{14}, the other must be H; R_3 is H or C_{1-12} alkyl; R_5 is any one of CHZ_{10}-$(CH_2)_{n1}$-V^2, CH_2-NH, CH_2-S, CH_2-CH_2, CH_2-CO, or CO-CH_2 (where V^2 is either of

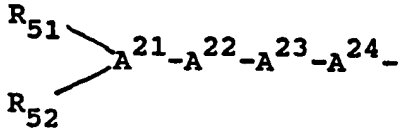


(where each R_7, R_8, and R_9 independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, or C_{12-20} naphthylalkyl); n1 is either 1 or 0; and Z_{10} is either of H or OH); R_6 is C or and V^1 is any of



or deleted (where each R_{10}, R_{11}, and R_{12} independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, or C_{12-20} naphthylalkyl); provided that, where R_5 is CHZ_{10}-$(CH_2)_{n1}$-V^2, A^{11}, R_6H, and V^1 must be deleted; further provided that, where A^{11}, R_6H, and V^1 are deleted, R_5 must be CHZ_{10}-$(CH_2)_{n1}$-V^2; further provided that, where A^5 is Asp, A^6 is Ser, A^7 is Phe, A^8 is Val, A^9 is Gly, A^{10} is Leu, A^{11} is Leu, and R_5 is CH_2NH, at least one of A^1, A^2, A^3, or A^4 must be present; or a pharmaceutically acceptable salt thereof.

Another class of peptide of the invention includes the substance P analogs of the formula:



$$-R_{53}H\text{-}R_{54}\text{-}\underset{\displaystyle A^{25}}{R_{55}}H\text{-}R_{56}\text{-}\underset{\displaystyle A^{26}}{R_{57}}H\text{-}R_{58}\text{-}\underset{\displaystyle A^{27}}{R_{59}}H\text{-}R_{60}\text{-}\underset{\displaystyle A^{28}}{R_{61}}H\text{-}R_{62}\text{-}\underset{\displaystyle A^{29}}{R_{63}}H\text{-}R_{64}\text{-}\underset{\displaystyle A^{30}}{R_{65}}H\text{-}V^{10}$$

wherein

A^{21} = the D or L isomer of any one of the amino acids Arg Lys, or Lys-ϵ-NH-R_{80} (where R_{80} is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_{20} (where E_{20} is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or C_1-C_{12} acyl); or is deleted;

A^{22} = the D or L isomer of the amino acid Pro; or is deleted;

A^{23} = the D or L isomer of any one of the amino acids Lys or Lys-ϵ-NH-R_{82} (where R_{82} is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_{22} (where E_{22} is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or C_1-C_{12} acyl); or is deleted;

A^{24} = the D or L isomer of the amino acid Pro; or is deleted;

A^{25} = the identifying group of the D or L isomer of any one of the amino acids Asp, Gln, ß-Nal, Trp, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3); or is deleted;

A^{26} = the identifying group of the D or L isomer of any one of the amino acids Arg, Sar, Pro, Gln, pGlu, Phe, Trp, cyclohexyl-Ala, or Asn;

A^{27} = the identifying group of the amino acid D-Trp; or the identifying group of the D or L isomer of any of Leu, Phe, or cyclohexyl-Ala; or is deleted;

A^{28} = the identifying group of the D or L isomer of any one of the amino acids Val, ß-Nal, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3); or is deleted;

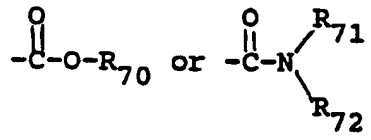
A^{29} = the identifying group of the amino acid D-Trp; or the identifying group of the D or L isomer of any of Leu, Phe, or cyclohexyl-Ala;

A^{30} = the identifying group of the D or L isomer of any one of the amino acids Leu, Nle, Ala, cyclohexyl-Ala, Val,

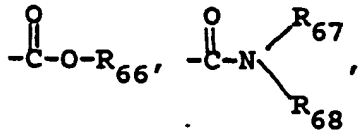
Ile, Met, Gly, Phe, Trp, ß-Nal, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3); or is deleted;

A^{31} = the identifying group of the D or L isomer of any one of the amino acids Trp, ß-Nal, Leu, Nle, Ala, Val, Ile, Met, Gly, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3); or is deleted;

each R_{51} and R_{52}, independently, is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_{24} (where E_{24} is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), C_1-C_{12} acyl, or is deleted, and R_{51} and R_{52} are bonded to the α-amino nitrogen atom of the N-terminal amino acid of said peptide, provided that, when one of R_{51} or R_{52} is COE_{24}, the other must be H; R_{53} is C, or is deleted; each R_{55}, and R_{61}, independently is C; each R_{57}, R_{59}, R_{63}, and R_{65}, independently, is either of C or deleted; R_{54} is CO-NH, $CO-NCH_3$, or is deleted; each R_{56} and R_{62}, independently, is any of CO-NH, $CHZ_{20}-(CH_2)_{n10}-CO-NH$ (where n10 is either 1 or 0; and Z_{20} is either of H or OH), CH_2-NH, CH_2-S, CH_2-CH_2, CH_2-CO, or $CO-CH_2$; R_{58} is any of $CO-NR_{69}$ (where R_{69} is H or C_{1-12} alkyl), $CHZ_{20}-(CH_2)_{n10}-CO-NH$, CH_2-NH, CH_2-S, CH_2-CH_2, CH_2-CO, $CO-CH_2$, or deleted; R_{60} is CO-NH, or deleted; R_{64} is any of CO-NH, $CHZ_{20}-(CH_2)_{n10}-V^{12}$, CH_2-NH, CH_2-S, CH_2-CH_2, CH_2-CO, $CO-CH_2$, or deleted (where V^{12} is either of



(where each R_{70}, R_{71}, and R_{72} independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, or C_{12-20} naphthylalkyl); and V^{10} is any of



or deleted (where each R_{66}, R_{67}, and R_{68} independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, or C_{12-20} naphthylalkyl); provided that, at least one of R_{56}, R_{58}, R_{62}, or R_{64} is other than either CO-NH or CO-NR_{69}; further provided that, where R_{56} is CHZ_{20}-$(CH_2)_{n10}$-CO-NH, A^{27}, $R_{57}H$, and R_{58} must be deleted; further provided that, where A^{27}, $R_{57}H$, and R_{58} are deleted, R_{56} must be CHZ_{20}-$(CH_2)_{n10}$-CO-NH; further provided that, where R_{58} is CHZ_{20}-$(CH_2)_{n10}$-CO-NH, A^{28}, $R_{59}H$, and R_{60} must be deleted; further provided that, where A^{28}, $R_{59}H$, and R_{60} are deleted, R_{58} must be CHZ_{20}-$(CH_2)_{n10}$-CO-NH; further provided that, where R_{62} is CHZ_{20}-$(CH_2)_{n10}$-CO-NH, A^{30}, $R_{63}H$, and R_{64} must be deleted; further provided that, where A^{30}, $R_{63}H$, and R_{64} are deleted, R_{62} must be CHZ_{20}-$(CH_2)_{n10}$-CO-NH; further provided that, where R_{64} is CHZ_{20}-$(CH_2)_{n10}$-V^{12}, A^{31}, $R_{65}H$, and V^{10} must be deleted; further provided that, where A^{31}, $R_{65}H$, and V^{10} are deleted, R_{64} must be CHZ_{20}-$(CH_2)_{n10}$-V^{12}; or a pharmaceutically acceptable salt thereof.

Examples of preferred peptides are: Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu$\psi[CH_2$-NH]Leu-NH_2; D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp$\psi[CH_2$-NH]Leu-Nle-NH_2; or D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leuψ [CH_2-NH]Nle-NH_2. (Non-peptide bonds in which the peptide bond is reduced are symbolized herein by "$\psi[CH_2$-NH]" or "ψ"].

In another aspect the invention features a substance P agonist of the formula: Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly$\psi[CH_2$-NH]Leu-Leu-NH_2.

SP antagonists of the invention are useful in the treatment of a patient suffering from diseases involving neurogenic inflamation e.g., rheumatoid arthritis, ulcerative colitis, eczema, and Crohn's disease. The SP antagonists of the invention are useful as antiproliferative agents e.g., in the treatment of small cell lung carcinoma or disorders involving the proliferation of fibroblasts. The antiproliferative properties of the SP antagonists of the invention also allow their use in the prevention of glial scarring (thus facilitating nerve

regeneration). The action of the antagonists of the invention on neurotransmission allow their use as nonopiate analgesics. Their use as nonopiate analgesics can permit the restoration of opiate response. The antagonists of the invention are also useful as antisecretory agents, acting e.g., on the salivary glands or on the pancreas.

In the generic formulas given above, when any of R_1, R_2, R_7-R_{13}, R_{51}, R_{52}, R_{66}-R_{68}, or R_{70}-R_{72} is an aromatic, lipophilic group, the in vivo activity can be long lasting, and delivery of the compounds of the invention to the target tissue can be facilitated.

The identifying group of an α-amino acid (for case of pyroglutamate, see below) is the atom or group of atoms, other than the α-carbonyl carbon atom, the α-amino nitrogen atom, or the H atom, bound to the asymmetric α-carbon atom. To illustrate by examples, the identifying group of alanine is CH_3, the identifying group of valine is $(CH_3)_2CH$, the identifying group of lysine is $H_3N^+(CH_2)_4$ and the identifying group of phenylalanine is $(C_6H_6)CH_2$. The identifying group of a ß- or γ-amino acid is the analagous atom or group of atoms bound to respectively, the ß- or the γ-carbon atom. Where the identifying group of an amino acid is not specified it may be α, ß, or γ. In the case of pyroglutamate the identifying group consists of $-NH-CO-CH_2-CH_2-$.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

We first briefly describe the drawings.

Drawings

Fig. 1 is a pair of graphs illustrating the effect of pseudopeptides of spantide, D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2, (left panel) and of SP, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2, (right panel) on SP- stimulated amylase release from pancreatic acini.

Fig. 2 is a graph showing the effect of Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leuψ[CH_2-NH]Leu-NH_2 on SP-stimulated amylase release from pancreatic ancini.

Fig. 3 is a pair of graphs illustrating the ability of various SP and spantide pseudopeptides to inhibit binding of ^{125}I-BH-substance P to pancreatic ancini.

Fig. 4 is a graph showing the ability of SP and spantide pseudopeptides to inhibit binding of ^{125}I-[Tyr^4] bombesin to pancreatic acini.

We now describe the structure, synthesis, and use of preferred embodiments of the invention.

Structure

The peptides of the invention all have modifications, e.g., a non-peptide bond in at least one of the indicated positions in which the carbon atom participating in the bond between two residues is reduced from a carbonyl carbon to a methylene carbon. The peptide bond reduction method which yields this non-peptide bond is described in Coy et al., U.S. patent application, Serial No. 879,348, assigned to the same assignee as the present application, hereby incorporated by reference. The peptides of the invention can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids such as the hydrohalic acids, e.g., hydrochloric acid, sulfuric acid, or phosphoric acid.

Synthesis of Substance P Analogs

The synthesis of the substance P antagonist Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leuψ[CH_2NH]Leu-NH_2 follows. Other substance P analogs can be prepared by making the appropriate modifications of the following synthetic method.

The first step is the preparation of the intermediate Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leuψ[CH_2-NH]Leu-benzhydrylamine resin, as follows.

Benzhydrylamine-polystyrene resin (Vega Biochemicals, Inc.) (0.97 g, 0.5 mmole) in the chloride ion form is placed in the reaction vessel of a Beckman 990B peptide synthesizer programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid (TFA) in methylene chloride (2 times for 1 and 25 min. each); (c) methylene chloride; (d) ethanol; (e) methylene chloride; and (f) 10% triethylamine in chloroform.

The neutralized resin is stirred with alpha-t-butoxycarbonyl(Boc)-leucine and diisopropylcarbodiimide (1.5 mmole each) in methylene chloride for 1 hour, and the resulting amino acid resin is then cycled through steps (a) to (f) in the above wash program. Boc-leucine aldehyde (1.25 mmoles), prepared by the method of Fehrentz and Castro, Synthesis, p. 676 (1983), is dissolved in 5 ml of dry dimethylformamide (DMF) and added to the resin TFA salt suspension followed by the addition of 100 mg (2 mmoles) of sodium cyanoborohydride (Sasaki and Coy, Peptides 8:119-121 (1987); Coy et al., id.). After stirring for 1 hour, the resin mixture is found to be negative to ninhydrin reaction (1 min.), indicating complete derivatization of the free amino group.

The following amino acids (1.5 mmole) are then coupled successively in the presence diisopropylcarbodiimide (1.5 mmole), and the resulting amino acid resin is cycled through washing/deblocking steps (a) to (f) in the same procedure as above: Boc-Gly (Boc-Gly is coupled as a 6M excess of the p-nitrophenylester), Boc-Phe, Boc-Phe, Boc-Gln, Boc-Gln, (Boc-Gln is coupled as a 6 M excess of the p-nitrophenylester), Boc-Pro, Boc-Lys, Boc-Pro, and Boc-Arg. The completed resin is then washed with methanol and air dried.

The resin described above (1.6 g, 0.5 mmole) is mixed with anisole (5 ml) and anhydrous hydrogen fluoride (35 ml) at

0°C and stirred for 45 min. Excess hydrogen fluoride is evaporated rapidly under a stream of dry nitrogen, and free peptide is precipitated and washed with ether. The crude peptide is dissolved in a minimum volume of 2 M acetic acid and purified on a column (2.5 x 90 cm) of Sephadex G-25 which is eluted with 2 M acetic acid, followed by preparative medium pressure chromatography on a column (1.5 x 45 cm) of Vydac C_{18} silica (10-15 μm) which is eluted with linear gradients of acetonitrile in 0.1% trifluoroacetic acid using an Eldex Chromatrol gradient controller (flow rate 1 ml/min). Where necessary, analogues are further purified by re-chromatography on the same column with slight modifications to the gradient conditions when necessary. Homogeneity of the peptides was assessed by thin layer chromatography and analytical reverse-phase high pressure liquid chromatography, and purity was 97% or higher. Amino acid analysis gave the expected amino acid ratios. The presence of the reduced peptide bond was demonstrated by fast atom bombardment mass spectrometry. Each of the analogues gave good recovery of the molecular ion corresponding to the calculated molecular mass.

A statine, AHPPA, ACHPA, ß-amino acid, or γ-amino acid residue is added in the same way as is a natural α-amino acid residue, by coupling as a Boc-amino acid. Statine or Boc-statine can be synthesized according to the method of Rich et al., 1978, J. Org. Chem. 43; 3624; Rich et al. (1988) J. Org. Chem. 53:869; and Rich et al., 1980, J. Med. Chem. 23:27. AHPPA can be synthesized according to the method of Hui et al., 1987, J. Med. Chem. 30:1287. ACHPA can be synthesized according to the method of Schuda et al., 1988, Journal of Organic Chemistry 53:873. Boc-coupled synthetic amino acids are avilable from Nova Biochemicals (Switzerland), Bachem (Torrance, California), and CalBiochem (San Diego, California).

Other compounds can be prepared as above and tested for effectiveness as agonists or antagonists in the following test program.

Phase 1- Amylase Release From Pancreatic Acini

SP stimulates the release of amylase in pancreatic acini. The stimulation or inhibition of release of amylase by pancreatic acini is used as a measure of, respectively, the agonist or antagonist activity of a peptide. Dispersed acini from the pancreas of one animal are suspended in 150 ml of standard incubation solution. Amylase release is measured as described previously (Gardner et al. (1977) J. Physiol. 270: 39). Amylase activity is determined by the methods of Ceska et al. (Ceska et al. (1969) Clin. Chim. Acta. 26:437 and Ceska et al. (1969) Clin. Chim. Acta. 26:445) using the Phadebas reagent. Amylase release is calculated as the percentage of the amylase activity in the acini at the beginning of the incubation that was released into the extracellular medium during the incubation.

Phase 2 - Competitive Inhibition of ^{125}I-Bolton-Hunter-SP Binding

Binding of ^{125}I-Bolton-Hunter-SP (^{125}I-BH-SP) to dispersed pancreatic acini is measured as described previously (Jensen et al. (1984) Biochem. Biophys. Acta. 804:181 and Jensen et al. (1988) Am. J. Physiol. 254:G883). Incubations contain 0.125 nM ^{125}I-BH-SP and 0.1% bacitracin in standard incubation buffer and were for 30 min at 37°C. Nonsaturable binding of ^{125}I-BH-SP is the amount of radioactivity associated with the acini when the incubation contains 0.125 nM ^{125}I-BH-SP plus 1 μM unlabeled SP. All values given are for saturable binding, i.e., binding measured with ^{125}I-BH-SP alone (total binding). In all experiments nonsaturable binding was < 30% of total binding.

Phase 3- Competitive Inhibition of ^{125}I-[Tyr^4] Bombesin Binding

^{125}I-[Tyr^4] Bombesin (2200 Ci/mmol) is prepared using a modification of the method described previously (Jensen et al. (1978) Proc. Natl. Acad. Sci. USA 75:6139). Iodo-Gen (mg) is dissolved in 5 ml of chloroform and 5 μl of this solution (1 μg Iodo-Gen) is transferred to a vial under a stream of nitrogen. To this vial 50 μl of KH_2PO_4 (pH 7.4), 6 μg [Tyr^4]bombesin in 5 μl water and 1 mCi Na^{125}I is added, mixed and incubated for 6 min

at 4°C, at which time the iodination mixture is added to a vial of 1 M dithiothreitol and incubated at 80°C for 60 min. The iodination mixture is then loaded onto a Sep-Pak cartridge and eluted with 0.25 M tetraethylammonium phosphate (TEAP) followed by 50% (vol/vol) acetonitrile-0.25 M TEAP. ^{125}I-[Tyr^4]bombesin is purified using reverse-phase high-performance liquid chromatography (HPCL) and eluted.

Results of Assays of Test Peptides

A number of analogues of substance P, or of the substance P antagonist spantide, each containing a non-peptide bond, can be synthesized and tested in one or more of the assays described in Phase 1 - Phase 3 above. The structure of substance P is Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met- NH_2. Spantide is an analog of SP. The structure of spantide is D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2. Stimulation or inhibition of the release of amylase from dispersed pancreatic acini was used as an assay for SP agonist activity or SP antagonist activity respectively. At a concentration of 10 μM, 9 of 10 SP and spantide derived pseudopeptides failed to stimulate amylase release when present alone (Table 2). One peptide, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe- Glyψ[CH_2-NH]Leu-Leu-NH_2, (10 μM) had agonist activity causing a 3-fold increase in amylase release (Table 2). Each of the 9 pseudopeptides without agonist activity was examined for activity as a SP antagonist. At a concentration of 10μM each of the spantide derived pseudopeptide analogues inhibited 1 nM-SP-stimulated amylase release. (Table 2) Three SP pseudopeptides, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leuψ[CH_2-NH]Leu-NH_2, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Pheψ[CH_2-NH]Gly-Leu-Leu-NH_2, and Arg-Pro-Lys-Pro-Gln-Glnψ[CH_2-NH]Phe-Phe-Gly-Leu-Leu- NH_2 caused inhibition (Table 2).

The relative abilities of each peptide to inhibit SP-stimulated amylase release was determined by the effect of peptide dose on inhibition. Dose-inhibition studies were carried out for each of the 9 pseudopeptides using a concentration of SP (1nM) that causes half-maximal stimulation. (Fig. 1). Results

for the 5 spantide derived pseudopeptides are shown in Fig. 1, left panel. D-Arg-Pro-Lys-Pro-Gln-Gln- D-Trp-Phe-D-Trp-Leu$\lfloor$[CH_2-NH]Nle-NH_2 was equipotent to spantide, causing detectable inhibition at 0.03 μM and half-maximal inhibition at 1.8 μM (Table 3). D-Arg-Pro-Lys- Pro-Gln-Gln-D-Trp-Phe-D-Trp$\lfloor$[CH_2-NH]Leu-Nle-NH_2 was 2-fold less potent (IC_{50} 3.5 μM, Table 3) than spantide. D-Arg-P -Lys-Pro-Gln-Gln$\lfloor$[CH_2-NH]D-Trp-Phe-D-Trp-Leu-Nle-NH_2 was 2.6-times (IC_{50} 4.7 μM) less potent than spantide. D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp$\lfloor$[CH_2-NH]Phe-D-Trp-Leu-Nle-NH_2 was 3.5-times (IC_{50}, 6.4-μM) less potent and D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe$\lfloor$[CH_2-NH]D-Trp-Leu-Nle-NH_2 was 17-times (IC_{50}, 30 μM) less potent than spantide (Table 3).

Results for the SP pseudopeptide analogues are shown in Fig. 1, right panel. Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu$\lfloor$[CH_2-NH]Leu-NH_2 was the most potent SP derivative causing detectible inhibition at 0.3 μM and half-maximal inhibition at 7.1 μM (Table 3, right). Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe$\lfloor$[CH_2-NH]Gly-Leu- Leu-NH_2 and Arg-Pro-Lys-Pro-Gln-Gln$\lfloor$[CH_2-NH]Phe-Phe-Gly-Leu-Leu-NH_2 were less effective, causing detectable inhibition at 10 μM. They are, respectively, 7-fold and 46-fold less potent than Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe- Gly-Leu$\lfloor$[CH_2-NH]Leu-NH_2 (Table 3, right). Arg-Pro-Lys-Pro-Gln-Gln-Phe$\lfloor$[CH_2-NH]Phe-Gly-Leu-Leu-NH_2 exhibited no inhibitory activity at concentrations as high as 30 μM (Fig. 1, right). The most potent SP derived pseudopeptide antagonist was Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe- Gly-Leu$\lfloor$[CH_2-NH]Leu-NH_2 (Fig. 1, Table 2).

The inhibitory effects of the most potent SP derived pseudopeptide antagonist, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu$\lfloor$[CH_2-NH]Leu-NH_2, are shown in Fig. 2. Acini were incubated with increasing concentrations of SP. Amylase release was detectible with 0.1 nM SP, was half-maximal with 1 nM SP2, and was maximal with 10 nM (Fig. 2). Addition of Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu$\lfloor$[CH_2-NH]Leu-NH_2 caused a parallel rightward shift in the dose-response curve for SP-stimulated amylase release. The shift was proportional to the concentration

of Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly- Leu$\psi[CH_2\text{-}NH]$Leu-NH_2 added but there was no change in the maximal response (Fig. 2). D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp- Phe-D-Trp-Leu$\psi[CH_2\text{-}NH]$Nle-NH_2 gave similar results (data not shown).

The interaction of SP and spantide derived analogues with the SP receptors of pancreatic acini was measured by the ability of a peptide to inhibit the binding of ^{125}I-BH-SP to acini. (See Table 3 and Fig. 3.)

The spantide derived pseudopeptides show a range of potencies. D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leuψ [CH_2NH]Nle-NH_2 is roughly equivalent in potency to spantide causing detectible inhibition at 0.03 μM and half-maximal inhibition at 2.2 μM (Fig. 3, left, Table 3). D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp$\psi[CH_2\text{-}NH]$Leu-Nle-NH_2 and D-Arg-Pro-Lys-Pro-Gln-Gln$\psi[CH_2\text{-}NH]$D-Trp-Phe-D-Trp- Leu-Nle-NH_2 are 2-fold less potent than spantide. D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp$\psi[CH_2\text{-}NH]$Phe-D-Trp-Leu-Nle-NH_2 was 3-times (K_i, 6.3 μM) less potent than spantide. D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe$\psi[CH_2\text{-}NH]$D-Trp-Leu-Nle-NH_2 was 7-times (K_i, 14.7 μM) less potent than spantide (Fig. 3, Table 3).

The SP derived pseudopeptides also show a range of potencies. Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu$\psi[CH_2\text{-}NH]$Leu-NH_2 causes detectable inhibition at 0.1 μM, and half-maximal inhibition at 3 μM. Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Glyψ [CH_2-NH]Leu-Leu-NH_2 is 1.5-fold lower in potency, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe$\psi[CH_2\text{-}NH]$Gly-Leu-Leu-NH_2 is 20-times less potent, and Arg-Pro-Lys-Pro-Gln-Gln-Pheψ [CH_2-NH]Phe-Gly-Leu-Leu-NH_2 and Arg-Pro-Lys-Pro-Gln- Gln$\psi[CH_2\text{-}NH]$Phe-Phe-Gly-Leu-Leu-NH_2 are more than 62-times less potent than Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu$\psi[CH_2\text{-}NH]$Leu-NH_2.

Unlike previously studied SP analogs the peptides of the invention are specific to the SP receptor. Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu$\psi[CH_2\text{-}NH]$Leu-NH_2 was tested for the ability to inhibit amylase release produced by various pancreatic secretagogues (Table 4). Arg-Pro-Lys-Pro-

Gln-Gln-Phe-Phe-Gly-Leu$^{\psi}$[CH_2-NH]Leu-NH_2 (20 μM) inhibited amylase release stimulated by SP, but did not alter bombesin, CCK-8, carbachol, VIP, secretin, CGRP A23187 or TPA stimulated amylase release.

The most potent receptor antagonists were tested for their ability to inhibit the binding of ^{125}I-[Tyr^4] bombesin to the bombesin receptors of pancreatic acini. Spantide inhibited binding of ^{125}I-[Tyr^4] bombesin as reported previously (Jensen et al. (1988) Am. J. Physiol. 254:G883) causing half-maximal inhibition at 3 ± 1 μM and complete inhibition at 100 μM (Fig. 4). D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu$^{\psi}$[CH_2-NH] Nle-NH_2 inhibited ^{125}I-[Tyr^4]bombesin binding but was 3-fold less potent than spantide causing half-maximal inhibition at 10 μM ($p < 0.05$ compared to spantide) (Fig. 4). Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu$^{\psi}$[CH_2-NH] Leu-NH_2 did not cause detectable inhibition until concentrations above 30 μM and had a calculated K_i of 300 ± 20 μM. Spantide and D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D- Trp-Leu$^{\psi}$[CH_2-NH]Nle-NH_2 had a 3- to 10-fold lower affinity for inhibiting ^{125}I- [Tyr^4]bombesin binding as compared to ^{125}I-BH-SP.

The ability of Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu$^{\psi}$ [CH_2-NH]Leu-NH_2 to inhibit binding of ^{125}I-[Tyr^4] bombesin was 70-fold lower than its ability to inhibit binding of ^{125}I-BH-SP.

Use

The peptides of the invention may be administered to a mammal, particularly a human, in one of the traditional modes (e.g., orally, parenterally, transdermally, or transmucosally), in a sustained release formulation using a biodegradable biocompatible polymer, or by on-site delivery using micelles, gels and liposomes.

The peptides can be administered to a human patient in a dosage of 0.5 μg/kg/day to 5 mg/kg/day.

Table 1. The five carboxyl-terminal residues of a variety of bioactive peptides.

Peptide	Carboxyl Terminal Sequence
Bombesin	-Val-Gly-His-Leu-Met-NH_2
Neuromedin-ß	-Thr-Gly-His-Phe-Met-NH_2
Neuromedin-C	-Val-Gly-His-Leu-Met-NH_2
Litorin	-Val-Gly-His-Phe-Met-NH_2
Neurokinin-A	-Phe-Val-Gly-Leu-Leu-NH_2
Neurokinin-B	-Phe-Val-Gly-Leu-Met-NH_2
Substance P	-Phe-Phe-Gly-Leu-Met-NH_2
Prototypical Tachykinin Sequence	-Phe-X-Gly-Leu-Met-NH_2 (where X = a branched aliphatic or aromatic amino acid residue)

Table 2. Effect of the various SP and spantide derived pseudopeptides on basal and SP-stimulated amylase release.

Peptide Added	Amylase Release (percent total) Alone	SP (1 nM)
None	3.4 ± 0.5	6.6 ± 0.7
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu$^{\psi}$[CH_2-NH]Leu-NH_2 (10μM)	3.1 ± 0.7	4.3 ± 0.5*
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly$^{\psi}$[CH_2-NH]Leu-Leu-NH_2 (10μM)	10.4 ± 1.0**	NT-agonist
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe$^{\psi}$[CH_2-NH]Gly-Leu-Leu-NH_2 (10μM)	3.1 ± 1.0	5.4 ± 0.7*
Arg-Pro-Lys-Pro-Gln-Gln-Phe$^{\psi}$[CH_2-NH]Phe-Gly-Leu-Leu-NH_2 (10μM)	3.8 ± 0.7	6.4 ± 0.3
Arg-Pro-Lys-Pro-Gln-Gln$^{\psi}$[CH_2-NH]Phe-Phe-Gly-Leu-Leu-NH_2 (10μM)	3.1 ± 0.9	6.1 ± 1.0*
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu$^{\psi}$[CH_2-NH]Nle-NH_2 (10μM)	3.8 ± 0.05	3.6 ± 0.1*
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp$^{\psi}$[CH_2-NH]Leu-Nle-NH_2 (10μM)	3.9 ± 0.8	4.2 ± 0.2*
D-Arg.Pro-Lys-Pro-Gln-Gln-D-Trp-Phe$^{\psi}$[CH_2-NH]D-Trp-Leu-Nle-NH_2 (10μM)	4.0 ± 0.5	5.6 ± 0.8*
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp$^{\psi}$[CH_2-NH]Phe-D-Trp-Leu-Nle-NH_2 (10μM)	4.1 ± 0.5	4.8 ± 1.2*
D-Arg-Pro-Lys-Pro-Gln-Gln$^{\psi}$[CH_2-NH]D-Trp-Phe-D-Trp-Leu-Nle-NH_2 (10μM)	3.8 ± 0.3	4.5 ± 0.8*

* Significantly less than SP alone $p < 0.05$

** Significantly greater than no additions $p < 0.01$

Acini were incubated at 37°C for 30 min with 1 nM SP and 10 μM concentrations of the various SP and spantide pseudopeptide analogues either alone or in combination. Amylase release was expressed as percent of amylase activity in acini at the start of incubation that was released into extracellular medium during incubation. Values are means ± 1SEM from at least 5 separated experiments. In each experiment, each value was determined in duplicate. Abbreviations: NT-agonist = Not tested as an antagonist because it was an agonist.

Table 3. Abilities of SP, spantide and pseudopeptide to inhibit binding of ^{125}I-BH-SP or SP-stimulated amylase release.

Peptide	125I-BH-sp Binding K_i or K_d (μM)	Inhibition of 1Nmsp-stimulated Amylase Release IC_{50} (μM)
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2 (Spantid	2.1 ± 0.6	1.8 ± 0.1
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leuψ[CH_2NH]Nle-NH_2	2.2 ± 0.4	1.8 ± 0.25
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trpψ[CH_2-NH]Leu-Nle-NH_2	3.6 ± 0.7	3.5 ± 0.6
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Pheψ[CH_2-NH]D-Trp-Leu-Nle-NH_2	14.7 ± 2.0	30 ± 5.0
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trpψ[CH_2-NH]Phe-D-Trp-Leu-Nle-NH_2	6.3 ± 3.3	6.4 ± 1.4
D-Arg-Pro-Lys-Pro-Gln-Glnψ[CH_2-NH]D-Trp-Phe-D-Trp-Leu-Nle-NH_2	4.3 ± 1.1	4.7 ± 1.3
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (SP)	0.0025 ± 0.0005	No-Agonist
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leuψ[CH_2-NH]Leu-NH_2	4.3 ± 0.3	7.1 ± 0.9*
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Glyψ[CH_2-NH]Leu-Leu-NH_2	5.6 ± 2.2	No-Agonist
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Pheψ[CH_2-NH]Gly-Leu-Leu-NH_2	41.3 ±16.7	>30
Arg-Pro-Lys-Pro-Gln-Gln-Pheψ[CH_2-NH]Phe-Gly-Leu-Leu-NH_2	265.0 ± 89.0	>30
Arg-Pro-Lys-Pro-Gln-Glnψ[CH_2-NH]Phe-Phe-Gly-Leu-Leu-NH_2	310.0 ± 88.0	>30

Values are means ± 1SEM. ^{125}I-Kd values for SP are obtained from Scatchard analysis of ^{125}I-labeled SP binding studies. K_i values for agonist or antagonists from studies of binding ^{125}I-BH-SP were obtained according to the equation: $K_i = (R/1-R)\ (SB/S+A)$ where R is the observed saturable binding of ^{125}I-BH-SP in the presence of antagonist (B) expressed as a fraction of that obtained when B is not present; A is the concentration of ^{125}I-BH-SP (0.125 nM). B is the concentration of antagonist, S is the K_d of SP determined by Scatchard analysis. No-agonist = peptide not tested for inhibition activity because agonist activity when present alone.

Table 4. Ability of Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-LeuΨ[CH_2-NH]Leu-NH_2 to affect amylase release stimulated by various secretagogues.

Secretagogue	Amylase Release (percent total) Alone	Leu^{11},Ψ10-11-SP (20 μM)
None	2.7 ± 0.4	2.3 ± 0.3
Substance P (1 nM)	8.7 ± 1.8	4.3 ± 0.5*
CCK-8 (0.1 nM)	19.7 ± 4.2	21.6 ± 4.9
Bombesin (0.3 nM)	14.8 ± 4.1	14.3 ± 3.1
Carbachol (10 μM)	22.7 ± 4.1	20.7 ± 4.3
VIP (0.3 nM)	20.5 ± 3.5	18.9 ± 4.2
Secretin (0.1 μM)	18.9 ± 3.6	18.8 ± 4.1
CGRP (0.1 μM)	12.1 ± 3.3	12.0 ± 3.9
A23187 (0.1 μM)	9.9 ± 1.5	11.6 ± 1.6
TPA (0.1 μM)	32.2 ± 5.7	30.2 ± 5.6

*Significantly less than secretagogue alone $p < 0.001$

Acini were incubated for 30 min at 37°C with various pancreatic secretagogues alone or with Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-LeuΨ[CH_2-NH]LeuNH_2. In each experiment, each value was determined in duplicate, and results give means ± 1SEM from at least 4 separated experiments. Abbreviations: CCK-8, COOH-terminal octapeptide of cholecystokinin; VIP, vasoactive intestinal peptide, respectively; CGRP, calcitonin gene-related peptide; A23187 and TPA, 1,2-o-tetradecanoylphorbol-1,3-acetate.

Other Embodiments

Other embodiments are within the following claims.

What is claimed is:

5

Claims

1. A linear peptide which is an analog of naturally occurring, biologically active substance P having an active site and a binding site responsible for the binding of said peptide to a receptor on a target cell, said analog having one of the following modifications, (a) a non-peptide bond instead of a peptide bond between an amino acid residue of said active site and an adjacent amino acid residue, (b) a statine or AHPPA or ACHPA or ß-amino acid or γ-amino acid residue in place of two naturally occurring amino acid residues of said active site, (c) a deletion of an amino acid residue within the active site and a modification of an amino acid residue outside the active site, or (d) the presence of an N-terminal amino acid residue that is not the natural N-terminal amino acid residue of said naturally occurring, biologically active peptide.

2. A linear peptide, which is an analog of substance P, of claim 1 wherein said analog is capable of binding to said receptor, so that said analog is capable of acting as a competitive inhibitor of said naturally occurring peptide by binding to said receptor and, by virtue of one of said modifications, failing to exhibit the in vivo activity of said naturally occurring peptide.

3. A linear peptide of claim 1 wherein said active site of said linear peptide is in the carboxyl terminal-half of said linear peptide, said linear peptide having one of the following modifications, (a) a non-peptide bond instead of a peptide bond between an amino acid residue of said active site and an adjacent amino acid residue, (b) a statine or AHPPA or ACHPA or ß-amino acid or γ-amino acid residue in place of two naturally occurring amino acid residues of said active site, (c) a deletion of an amino acid residue within the active site and a modification of an amino acid residue outside the active site, or (d) the presence of an N-terminal amino acid residue that is not

the natural N-terminal amino acid residue of said naturally occurring, biologically active peptide.

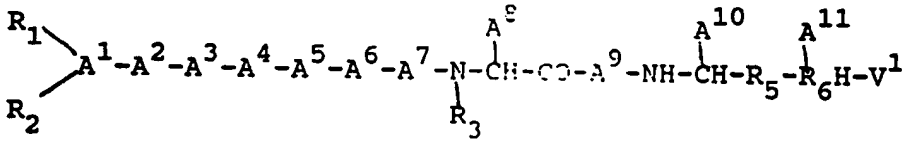
4. A linear peptide of claim 1 wherein said active site of said linear peptide is in the carboxyl terminal-half of said linear peptide, said linear peptide having one of the following modifications, (a) a non-peptide bond instead of a peptide bond between the carboxyl terminal amino acid residue and the adjacent amino acid residue, or (b) a statine or AHPPA or ACHPA or ß-amino acid or γ-amino acid residue in place of the naturally occurring carboxyl terminal and adjacent amino acid residues.

5. A linear peptide of claim 1 wherein said linear peptide is an analog of naturally occurring, biologicall active substance P, said analog having one of the following modifications, (a) a non-peptide bond instead of a peptide bond between an amino acid residue of said active site and an adjacent amino acid residue, or (b) a statine or AHPPA or ACHPA or ß-amino acid or γ-amino acid residue in place of two naturally occurring amino acid residues of said active site.

6. The substance P analog of claim 5 wherein said analog is at least 25% homologous with naturally occurring substance P.

7. The substance P analog of claim 5 wherein said analog is at least 50% homologous with naturally occurring substance P.

8. The substance P analog of claim 5 of the formula:



wherein

A^1 = the D or L isomer of any one of the amino acids Arg, Lys, or Lys-ε-NH-R_{20} (where R_{20} is any of H, C_{1-12}

alkyl, C_{7-10} phenylalkyl, COE_{10} (where E_{10} is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or C_1-C_{12} acyl); or is deleted;

A^2 = the D or L isomer of the amino acid Pro; or is deleted;

A^3 = the D or L isomer of any one of the amino acids Lys, or Lys-ϵ-NH-R_{22} (where R_{22} is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_{12} (where E_{12} is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or C_1-C_{12} acyl); or is deleted;

A^4 = the D or L isomer of the amino acid Pro; or is deleted;

A^5 = the D or L isomer of any one of the amino acids Asp, Gln, ß-Nal, Trp, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3); or is deleted;

A^6 = the D or L isomer of any one of the amino acids Ala, Arg, Ser, Pro, Gln, pGlu, Asn, ß-Nal, Trp, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3);

A^7 = the D or L isomer of any one of the amino acids Val, Thr, Phe, Trp, ß-Nal, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3);

A^8 = the identifying group of the D or L isomer of any one of the amino acids Gly, Val, Trp, ß-Nal, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3);

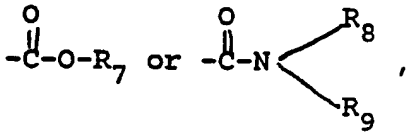
A^9 = the D or L isomer of any one of the amino acids Sar, His, Gly, Trp, ß-Nal, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3);

A^{10} = the identifying group of the D or L isomer of any one of the amino acids Trp, ß-Nal, Leu, Nle, Ala, cyclohexyl-Ala, Val, Ile, Met, Gly, Phe, o-X-Phe

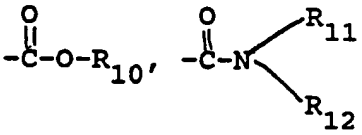
(where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3);

A^{11} = the identifying group of the D or L isomer of any one of the amino acids Trp, ß-Nal, Leu, Nle, Ala, Val, Ile, Met, Gly, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3); or is deleted;

each R_1 and R_2, independently, is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_{14} (where E_{14} is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), C_1-C_{12} acyl, or is deleted, and R_1 and R_2 are bonded to the α-amino nitrogen atom of the N-terminal amino acid of said peptide, provided that, when one of R_1 or R_2 is COE_{14}, the other must be H; R_3 is H or C_{1-12} alkyl; R_5 is any one of CHZ_{10}-$(CH_2)_{n1}$-V^2, CH_2-NH, CH_2-S, CH_2-CH_2, CH_2-CO, or CO-CH_2 (where V^2 is either of

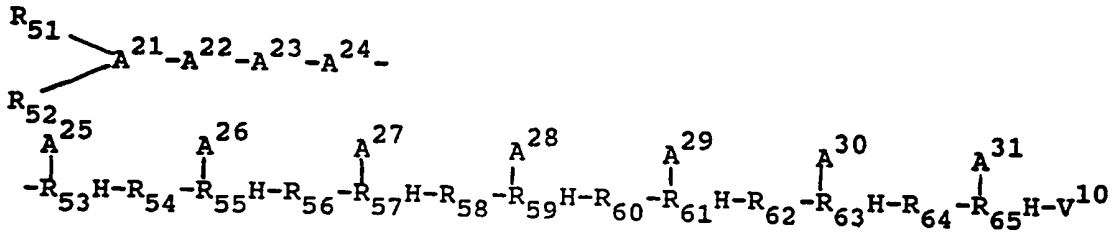


(where each R_7, R_8, and R_9 independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, or C_{12-20} naphthylalkyl); n1 is either 1 or 0; and Z_{10} is either of H or OH); R_6 is C or and V^1 is any of



or deleted (where each R_{10}, R_{11}, and R_{12} independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, or C_{12-20} naphthylalkyl); provided that, where R_5 is CHZ_{10}-$(CH_2)_{n1}$-V^2, A^{11}, R_6H, and V^1 must be deleted; further provided that, where A^{11}, R_6H, and V^1 are deleted, R_5 must be CHZ_{10}-$(CH_2)_{n1}$-V^2; further provided that, where A^5 is Asp, A^6 is Ser, A^7 is Phe, A^8 is Val, A^9 is Gly, A^{10} is Leu, A^{11} is Leu, and R_5 is CH_2NH, at least one of A^1, A^2, A^3, or A^4 must be present; or a pharmaceutically acceptable salt thereof.

9. The substance P analog of claim 5 of the formula:



wherein

A^{21} = the D or L isomer of any one of the amino acids Arg Lys, or Lys-ϵ-NH-R_{80} (where R_{80} is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_{20} (where E_{20} is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or C_1-C_{12} acyl); or is deleted;

A^{22} = the D or L isomer of the amino acid Pro; or is deleted;

A^{23} = the D or L isomer of any one of the amino acids Lys or Lys-ϵ-NH-R_{82} (where R_{82} is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_{22} (where E_{22} is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or C_1-C_{12} acyl); or is deleted;

A^{24} = the D or L isomer of the amino acid Pro; or is deleted;

A^{25} = the identifying group of the D or L isomer of any one of the amino acids Asp, Gln, ß-Nal, Trp, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3); or is deleted;

A^{26} = the identifying group of the D or L isomer of any one of the amino acids Arg, Sar, Pro, Gln, pGlu, Phe, Trp, cyclohexyl-Ala, or Asn;

A^{27} = the identifying group of the amino acid D-Trp; or the identifying group of the D or L isomer of any of Leu, Phe, or cyclohexyl-Ala; or is deleted;

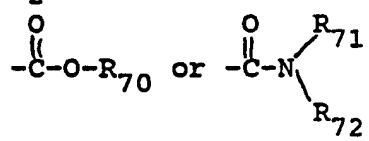
A^{28} = the identifying group of the D or L isomer of any one of the amino acids Val, ß-Nal, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3); or is deleted;

A^{29} = the identifying group of the amino acid D-Trp; or the identifying group of the D or L isomer of any of Leu, Phe, or cyclohexyl-Ala;

A^{30} = the identifying group of the D or L isomer of any one of the amino acids Leu, Nle, Ala, cyclohexyl-Ala, Val, Ile, Met, Gly, Phe, Trp, ß-Nal, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3); or is deleted;

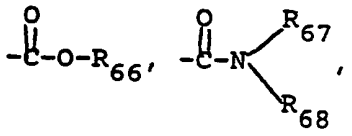
A^{31} = the identifying group of the D or L isomer of any one of the amino acids Trp, ß-Nal, Leu, Nle, Ala, Val, Ile, Met, Gly, Phe, o-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3), or p-X-Phe (where X = F, Cl, Br, NO_2, OH, or CH_3); or is deleted;

each R_{51} and R_{52}, independently, is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_{24} (where E_{24} is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), C_1-C_{12} acyl, or is deleted, and R_{51} and R_{52} are bonded to the α-amino nitrogen atom of the N-terminal amino acid of said peptide, provided that, when one of R_{51} or R_{52} is COE_{24}, the other must be H; R_{53} is C, or is deleted; each R_{55}, and R_{61}, independently is C; each R_{57}, R_{59}, R_{63}, and R_{65}, independently, is either of C or deleted; R_{54} is CO-NH, $CO-NCH_3$, or is deleted; each R_{56} and R_{62}, independently, is any of CO-NH, $CHZ_{20}-(CH_2)_{n10}-CO-NH$ (where n10 is either 1 or 0; and Z_{20} is either of H or OH), CH_2-NH, CH_2-S, CH_2-CH_2, CH_2-CO, or $CO-CH_2$; R_{58} is any of $CO-NR_{69}$ (where R_{69} is H or C_{1-12} alkyl), $CHZ_{20}-(CH_2)_{n10}$-CO-NH, CH_2-NH, CH_2-S, CH_2-CH_2, CH_2-CO, $CO-CH_2$, or deleted; R_{60} is CO-NH, or deleted; R_{64} is any of CO-NH, $CHZ_{20}-(CH_2)_{n10}-V^{12}$, CH_2-NH, CH_2-S, CH_2-CH_2, CH_2-CO, $CO-CH_2$, or deleted (where V^{12} is either of



,

(where each R_{70}, R_{71}, and R_{72} independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, or C_{12-20} naphthylalkyl); and V^{10} is any of



or deleted (where each R_{66}, R_{67}, and R_{68} independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, or C_{12-20} naphthylalkyl); provided that, at least one of R_{56}, R_{58}, R_{62}, or R_{64} is other than either CO-NH or CO-NR_{69}; further provided that, where R_{56} is CHZ_{20}-$(CH_2)_{n10}$-CO-NH, A^{27}, $R_{57}H$, and R_{58} must be deleted; further provided that, where A^{27}, $R_{57}H$, and R_{58} are deleted, R_{56} must be CHZ_{20}-$(CH_2)_{n10}$-CO-NH; further provided that, where R_{58} is CHZ_{20}-$(CH_2)_{n10}$-CO-NH, A^{28}, $R_{59}H$, and R_{60} must be deleted; further provided that, where A^{28}, $R_{59}H$, and R_{60} are deleted, R_{58} must be CHZ_{20}-$(CH_2)_{n10}$-CO-NH; further provided that, where R_{62} is CHZ_{20}-$(CH_2)_{n10}$-CO-NH, A^{30}, $R_{63}H$, and R_{64} must be deleted; further provided that, where A^{30}, $R_{63}H$, and R_{64} are deleted, R_{62} must be CHZ_{20}-$(CH_2)_{n10}$-CO-NH; further provided that, where R_{64} is CHZ_{20}-$(CH_2)_{n10}$-V^{12}, A^{31}, $R_{65}H$, and V^{10} must be deleted; further provided that, where A^{31}, $R_{65}H$, and V^{10} are deleted, R_{64} must be CHZ_{20}-$(CH_2)_{n10}$-V^{12}; or a pharmaceutically acceptable salt thereof.

10. The substance P analog of claim 9 of the formula: Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leuψ[CH_2-NH]Leu-NH_2;

11. The substance P analogs of claim 10 of the formula:

D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe- D-Trpψ[CH_2-NH]Leu-Nle-NH_2; or

D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp- Leuψ[CH_2-NH]Nle-NH_2.

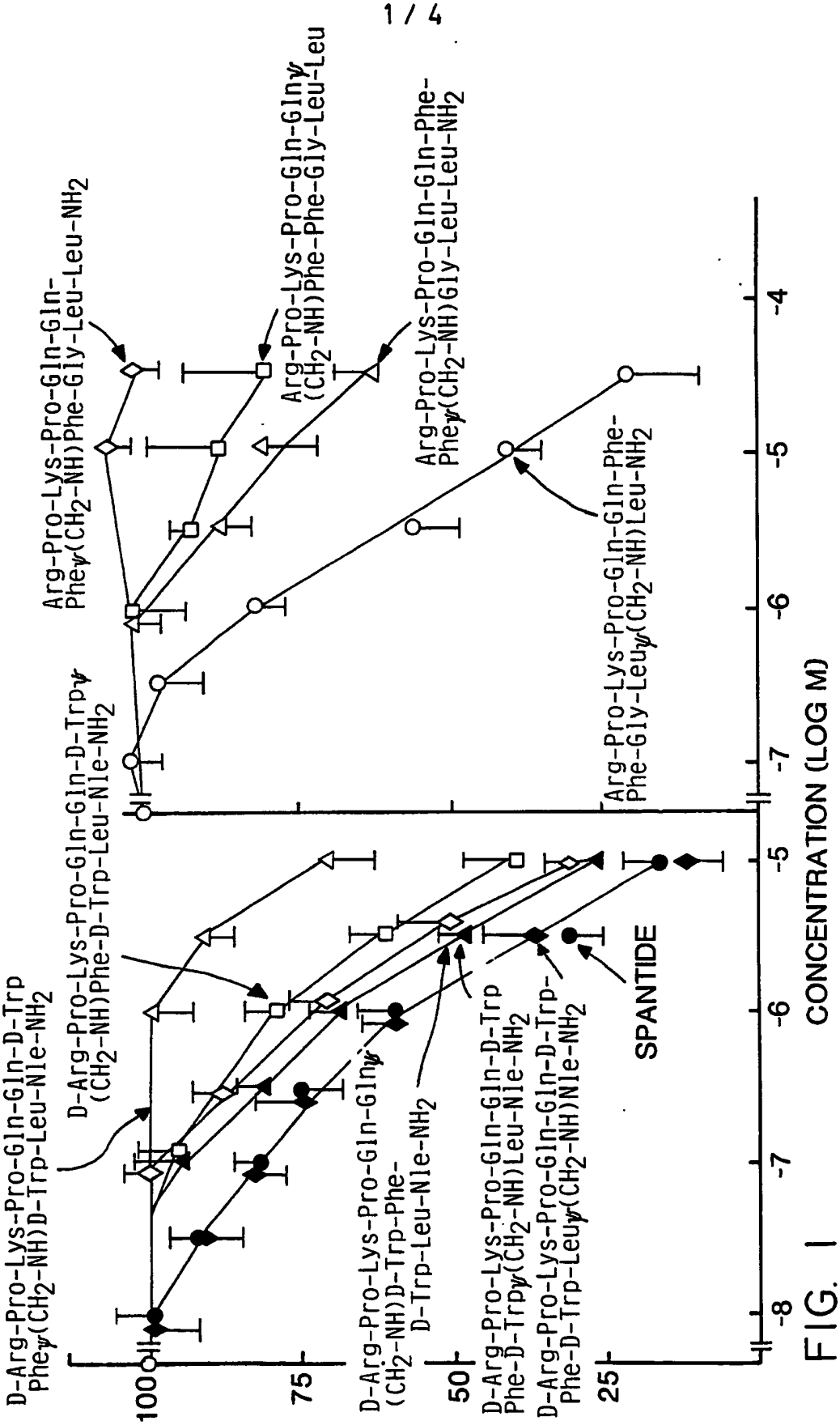
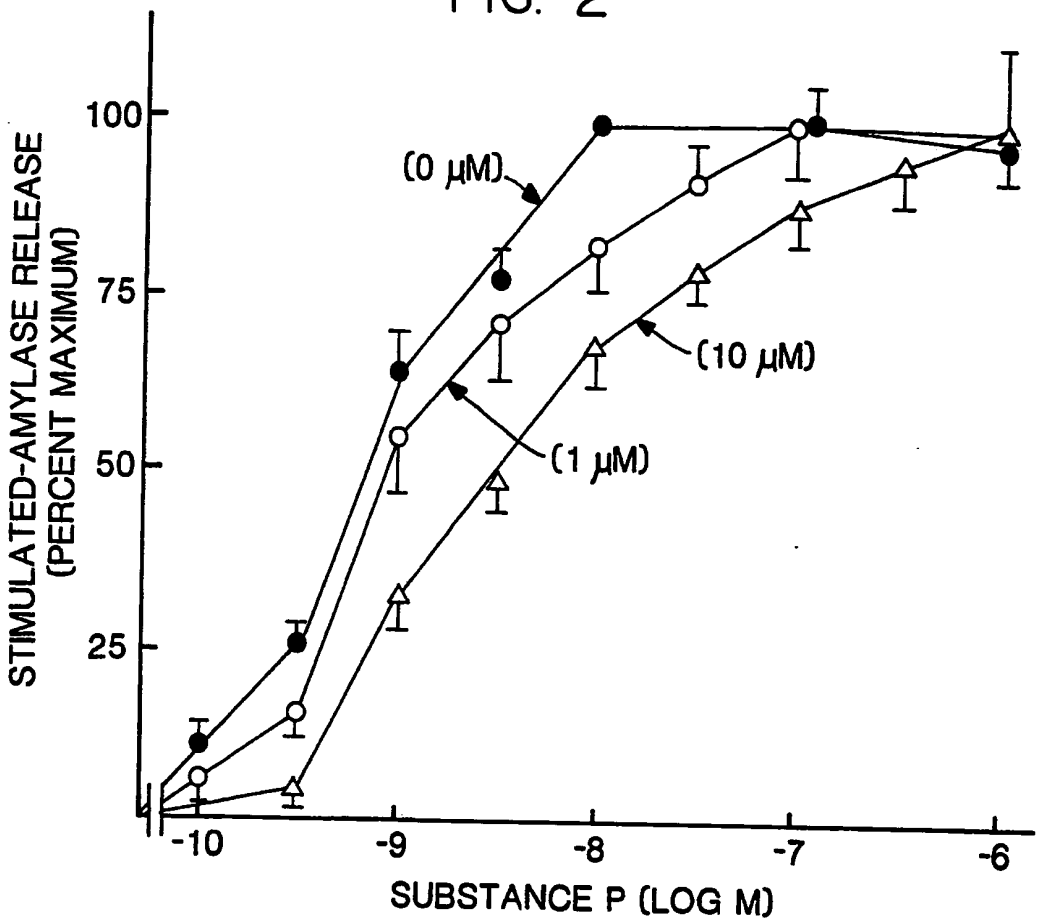

SP-STIMULATED AMYLASE RELEASE (PERCENT CONTROL)
100
75
50
25
-8
-7
-6
-5
-4
CONCENTRATION (LOG M)
SPANTIDE
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp Phe-D-Trpψ(CH2-NH)Leu-Nle-NH2
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp Phe-D-Trp-Leuψ(CH2-NH)Nle-NH2
D-Arg-Pro-Lys-Pro-Gln-Glnψ (CH2-NH)D-Trp-Phe- D-Trp-Leu-Nle-NH2
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp Phe ψ(CH2-NH)D-Trp-Leu-Nle-NH2
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trpψ (CH2-NH)Phe-D-Trp-Leu-Nle-NH2
Arg-Pro-Lys-Pro-Gln-Gln- Pheψ(CH2-NH)Phe-Gly-Leu-Leu-NH2
Arg-Pro-Lys-Pro-Gln-Glnψ (CH2-NH)Phe-Phe-Gly-Leu-Leu
Arg-Pro-Lys-Pro-Gln-Gln-Phe- Pheψ(CH2-NH)Gly-Leu-Leu-NH2
Arg-Pro-Lys-Pro-Gln-Gln-Phe- Phe-Gly-Leuψ(CH2-NH)Leu-NH2

FIG. 1

FIG. 2


STIMULATED-AMYLASE RELEASE
(PERCENT MAXIMUM)
100
75
50
25
(0 μM)
(10 μM)
(1 μM)
-10
-9
-8
-7
-6
SUBSTANCE P (LOG M)

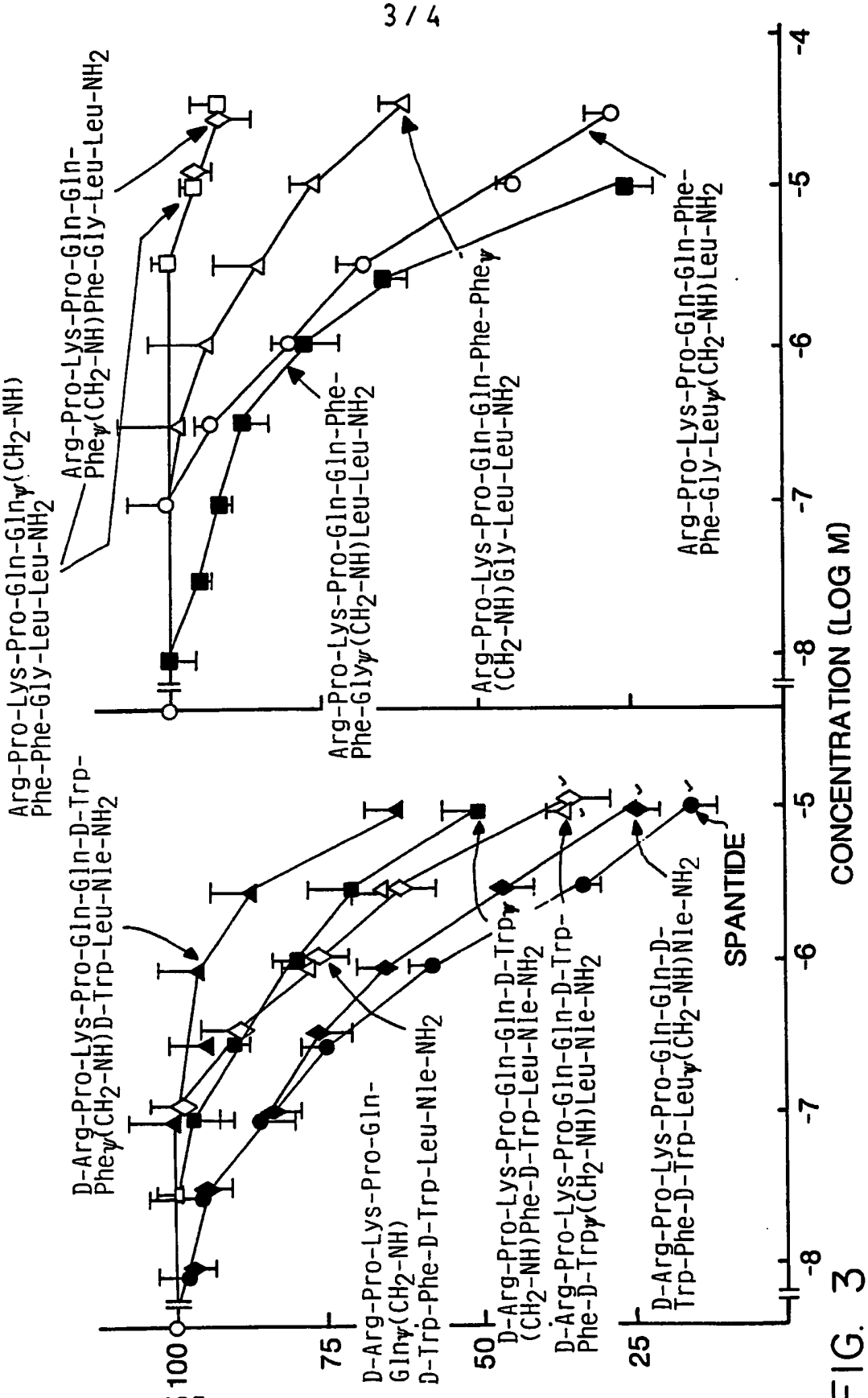

Arg-Pro-Lys-Pro-Gln-Glnψ(CH2-NH)
Phe-Phe-Gly-Leu-Leu-NH2
Arg-Pro-Lys-Pro-Gln-Gln-Gln-
Pheψ(CH2-NH)Phe-Gly-Leu-Leu-NH2
Arg-Pro-Lys-Pro-Gln-Gln-Phe-
Pheψ(CH2-NH)Leu-Leu-NH2
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Pheψ
(CH2-NH)Gly-Leu-Leu-NH2
Arg-Pro-Lys-Pro-Gln-Gln-Phe-
Phe-Glyψ(CH2-NH)Leu-Leu-NH2
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-
Pheψ(CH2-NH)D-Trp-Leu-Nle-NH2
D-Arg-Pro-Lys-Pro-Gln-
Glnψ(CH2-NH)
D-Trp-Phe-D-Trp-Leu-Nle-NH2
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trpψ
(CH2-NH)Phe-D-Trp-Leu-Nle-NH2
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-
Phe-D-Trpψ(CH2-NH)Leu-Nle-NH2
D-Arg-Pro-Lys-Pro-Gln-Gln-D-
Trp-Phe-D-Trp-Leuψ(CH2-NH)Nle-NH2
SPANTIDE
125I-BI SP BOUND (PERCENT CONTROL)
100
75
50
25
-4
-5
-6
-7
-8
CONCENTRATION (LOG M)

FIG. 3

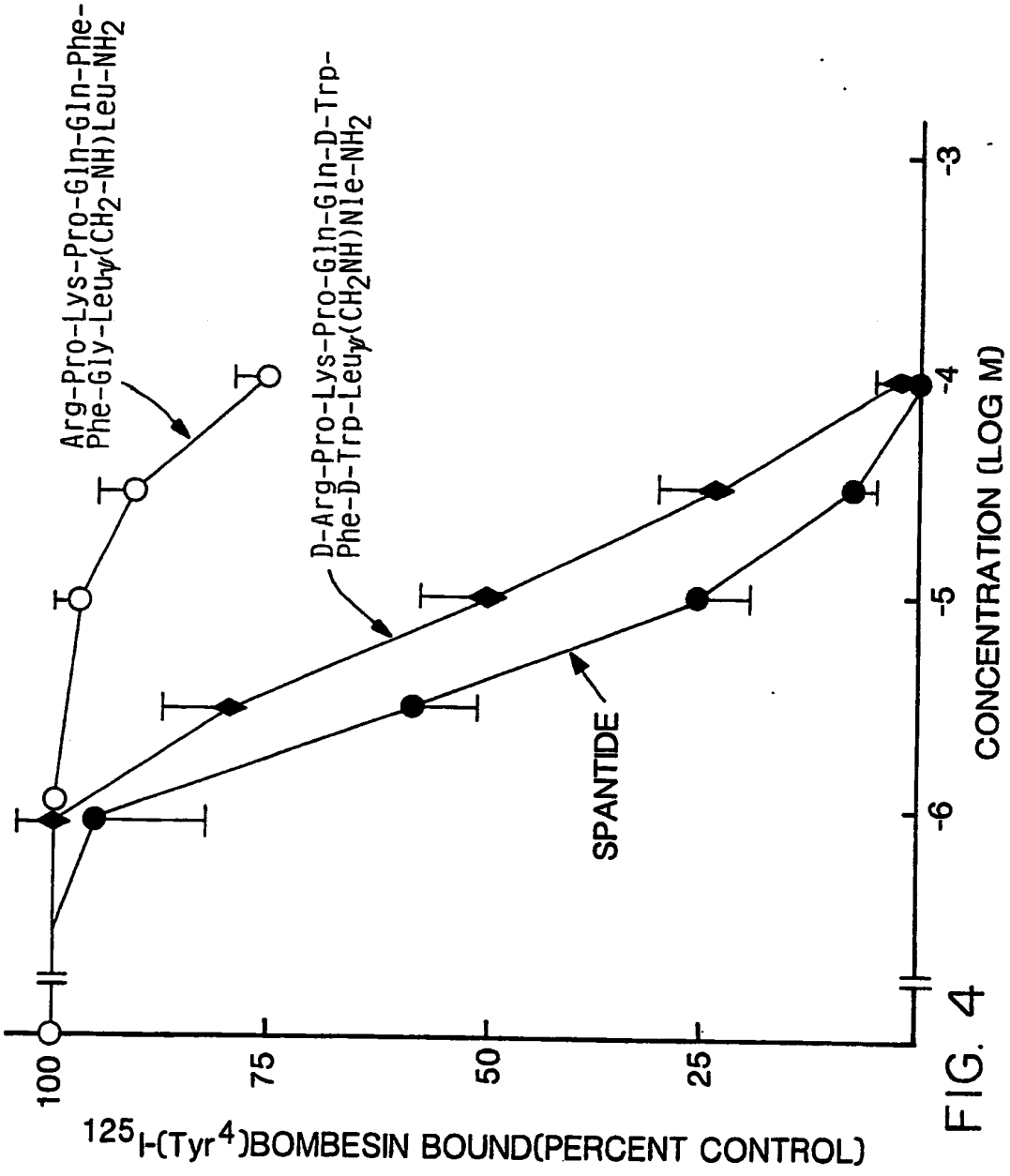

Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leuψ(CH2-NH)Leu-NH2
D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leuψ(CH2NH)Nle-NH2
SPANTIDE
100
75
50
25
-3
-4
-5
-6
CONCENTRATION (LOG M)
125I-(Tyr4)BOMBESIN BOUND(PERCENT CONTROL)

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/04633

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5):· C07K 5/02,5/06,5/08,5/10,7/02,7/06,7/08

U.S.CL.: 530/323,327,328,329,330,331,332,345

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	530/323,327,328,329,330,331,332,345

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

CHEMICAL ABSTRACTS AND BIOLOGICAL ABSTRACTS ONLINE COMPUTER SEARCH

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	US, A, 4,439,360 (Verdini et al.) 27 March 1984. See the abstract in particular.	1-7
X Y	US, A, 4,481,139 (Folkers et al.) 06 November 1984. See column 1, lines 24-39 and Table I in particular.	1-3 1-11
X	US, A, 3,862,114 (Scandrett) 21 January 1975. See Table I in particular.	1-3
Y	US, A, 4,803,261 (Coy et al.) 07 February, 1989. See column 3, lines 7-9 and claim 5 in particular.	1-11

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
14 September 1990	09 JAN 1991
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Christina Chan Christina Chan

Form PCT/ISA/210 (second sheet) (May 1986)

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